Synopsis of the proposed Ph.D. programme in Chemistry

Synthesis, characterization and antimicrobial evaluation of isoxazole substituted heterocycles

Submitted to

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ABSTRACT

1. Synthesis of novel imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo-[2,3-*a*]pyrimidin-10-ones **(5)** would be accomplished by reaction of 5-amino-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides **(2)**, with chloroacetyl chloride followed by treatment with aromatic primary amines and then with formaldehyde. The key intermediate *viz.,* 3-amino-5-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl-cyanides **(2)** was obtained by reaction of 3-amino-5-methyisoxazole **(1)** with aromatic aldehydes and malononitrile by a three-component one-pot process. The structures of newly synthesized compounds **(5)** will be established on the basis of spectral (IR, ^{1}H NMR, ^{13}C NMR, and Mass) and by micro analytical data. Compounds **(5)** will be evaluated for their *in vitro* antibacterial and antifungal data.

2. Synthesis of novel 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triaryl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitriles **(5**) would be achieved by the reaction of 5-amino-2-methyl-7-aryl-7*H*-isoxazolo-[2,3-*a*]pyrimidin-6-yl cyanides **(1)** with triethyl orthoformate, followed by cyclization with hydrazine hydrate to give compounds **(3)**. The reaction of **3** with benzoyl acteonitrile, followed by a three-component reaction with aromatic aldehydes and benzoyl acetonitrile in presence of $FeCl_3$ / Al_2O_3 affords the title compounds **(5)**. The structures of newly synthesized compounds **(2-5)** would be characterized by spectral and micro analytical data and evaluated for their antimicrobial activity.

Introduction:

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents has prompted studies on the development of new potential antimicrobial compounds. Isoxazole nucleus is a very important system in the field of new drug discovery, especially in the area of antimicrobials and antibiotics. Isoxazoles have been repeatedly shown as useful synthons in organic synthesis[1]. Among the isoxazole sulphanilamides, 5-methyl-3-isoxazolyl sulphanilamide under the trade name-*sulphamethoxazole*, is being widely used against a variety of bacterial infections[2]. The Penicillin derivative, consisting 3-(*o*-chlorophenyl) -5-methyl-isoxazolyl-4-carboxamide group known as "*Cloxacillin*" is used as a powerful antibiotic[3]. 5-Methylisoxazole derivatives are found to possess antibacterial and plant growth regulative activities[4]. Isoxazole containing orally active cephalosporin esters are reported to possess antibiotic activity[5]. A large number of isoxazole derivatives exhibited antibacterial, antifungal[6], anticonvulsant[7], analgesic[8], and anticancer [9, 10] activities.

Review of literature and development in the subject:

Heterocycles play a vital role in pharmacological, agricultural and synthetic fields[11]. Consequently the development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities. Isoxazole unit constitutes an easily accessible nucleus that is present in a number of natural and pharmacological compounds[12-17] and display a wide range of biological activity. Survey of literature revealed that when one biodynamic heterocyclic system was coupled with another, a molecule with enhanced biological activity[18,19] was produced. The chemistry of these linked heterocyclics has been a fascinating field of investigation in medicinal chemistry, as they have been found to exhibit enhanced biological profile[20].

The review of literature concerned to isoxazole revealed that isoxazole coupled heterocycles are displaying better activity. Many isoxazole substituted heterocycles have been synthesized and studied for their pharmacological activity. From the review, it is clear that a number of isoxazole derivatives are finding applicability in the chemotherapy. Synthesis and biological evaluation of isoxazole substituted heterocycles is a fast developing area, hence we are fascinated to synthesize a number of isoxazolyl heterocycles to evaluate their antimicrobial properties.

Previous work:

1. A new series of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]oxazines have been synthesized by condensation of 4-amino-3,5-dimethylisoxazole with salicylaldehydes followed by reduction with sodium borohydride and subsequent ring closure with formaldehyde by internal Mannich reaction. The newly synthesized title compounds were characterized by the spectral (IR, ^{1}H NMR, ^{13}C NMR, Mass) and by micro analytical data and were evaluated for their antimicrobial activity. Some of the compounds exhibited significant antimicrobial activity.

This work is published in "World journal of pharmacy and pharmaceutical sciences" vol. 6, issue 11, 1248-1258.

2. Synthesis of novel 5-(1-*H*-benzo[*d*]imidazol-2-yl)methyl)-3-(3,5-dimethyl-4-isoxazolyl)-2-aryl-thiazolidin-4-ones have been accomplished by a simple synthetic protocol. Condensation of 4-benzalamino-3,5-dimethylisoxazole with mercapto succinic acid, followed by cyclization with 1,2-phenylene diamines produced the title compounds. The structures of the compounds have been established by IR, ^{1}H NMR, ^{13}C NMR and mass and by elemental analyses. The title compounds have screened for their anti-inflammatory and analgesic activity, which showed promising results.

This work is published in "Indian journal of chemistry" sec-B, vol. 59B, 2020. 252-257.

Objectives of the research:

The main objective of the proposed research work is based on the new methodologies for the synthesis of isoxazolyl heterocylces and evaluate for their antibacterial and antifungal activity. As majority of the exhibiting drugs are resistant to bacteria and fungi, we predict that the newly synthesized isoxazolyl heterocyles many act as good bacteriocides and anti fungicides with good efficacy and no toxicity. All the newly synthesized isoxazlolyl heteroclycles will be characterized thoroughly by spectral (IR, ^{1}H NMR, ^{13}C NMR and mass) and micro analytical data. The new heterocyclics entities containing isoxazole may exhibit interesting pharmacological activity.

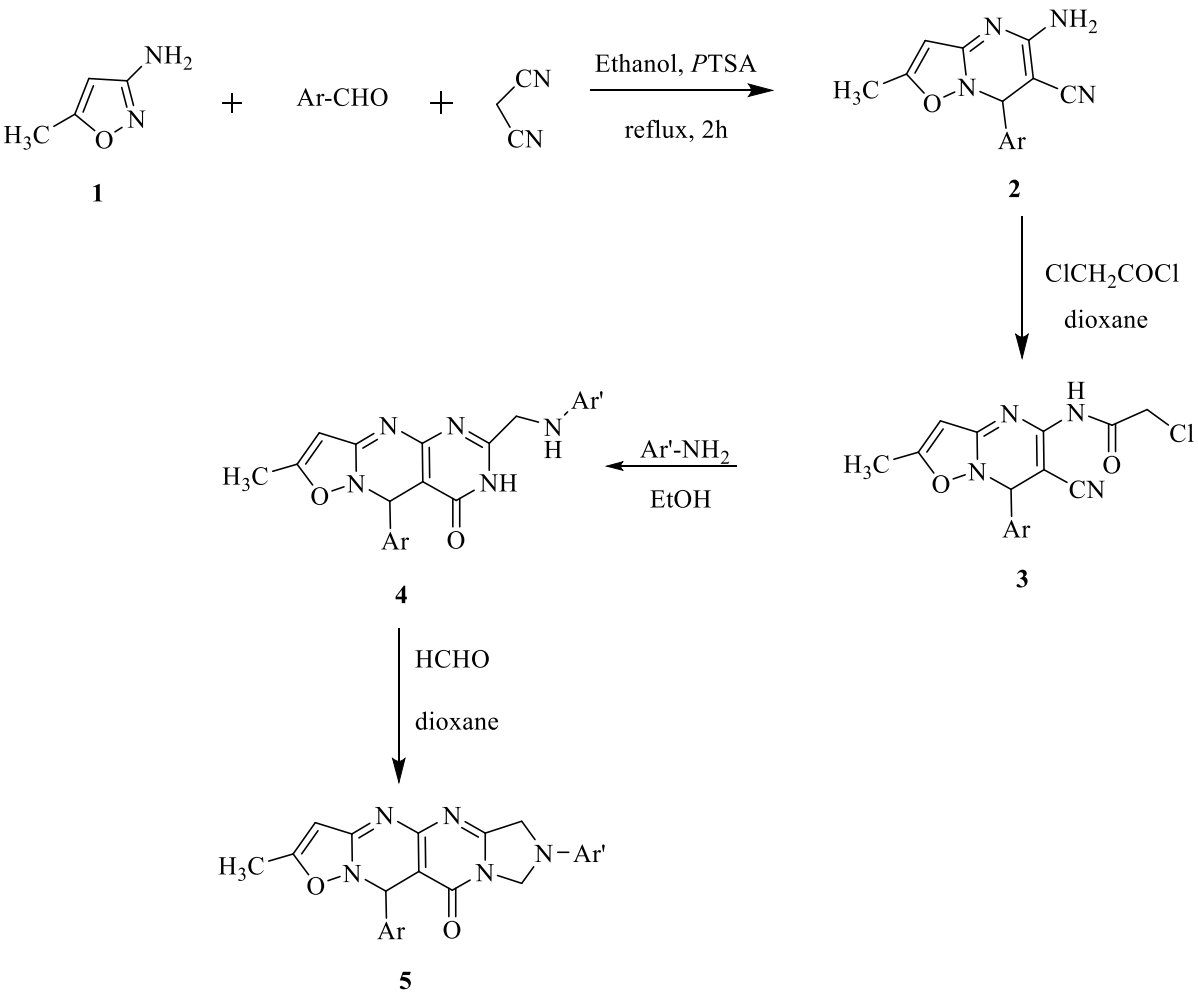
Research methodology to be adopted:

We will adopt new synthetic methodologies for the synthesis if isoxazolyl heteroclycles by utilizing readily available starting materials. The obvious advantages of our method are i) operational simplicity ii) excellent yields and iii) products are isolated in pure form by recrystallization without intervention of chromatography. This ease of purification compliments this synthetic technologies practical, easy to perform, and facile.

Method of Synthesis:

The three-component reaction of 3-amino-5-methylisoxazole (1), substituted aromatic aldehydes, and malononitrile in presence of *p*-toluene sulphonic acid (*P*TSA), a Lewis acid catalyst, in ethanol furnishes 5-amino-2-methyl-7-aryl-7*H*-isoxazolo [2,3-a]pyrimidin-6-yl cyanides (2). The reaction of 2 with chloroacetyl chloride in dioxane produces 2-chloro-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl) acetamides (3). Reaction of 3 with aromatic primary amines affords 8-methyl-5-aryl-2-((arylamino)methyl)-3,5-dihydro-4*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-ones (4). This reaction proceeds by nucleophilic substitution of the chloride ion with the primary amino group followed by Dimorth rearrangement in presence of excess of amine. Treatment of 4 with formaldehyde in dioxane yields 2-methyl-7,11-diaryl-6,7,8,11-tetrahydro-10*H*-imidazo[1',5':1,2]pyrimido [4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones (5). The structures of the compounds 2-5 will be determined by spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) and micro analytical data. The newly synthesized compounds will be evaluated for their antimicrobial activity.

Proposed Work: 1



Scheme I. Synthesis of imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones.

1. The reaction of 5-amino-2-methyl-7-aryl-7*H*-isoxazolo-[2,3-*a*]pyrimidin-6-yl cyanides **1** with triethyl orthoformate under refluxing condition produce the corresponding ethyl-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidates **(2)** in good yields. Compounds **2** on heating with hydrazine hydrate affords the cyclized products *viz.,* 4-hydrazinyl-5-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines **3**. The reaction of **3** with benzoyl acetonitrile in refluxing ethanol underwent cyclization to yield 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-aryl-1*H*-pyrazol-5-amines **(4)**. The synthesis of title compounds *viz.,* 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triaryl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitriles **(5**) would be achieved by trimolecular condensation of **4** with aromatic aldehydes and benzoyl acetonitrile in presence of $FeCl_3$ / basic Al_2O_3 in anhydrous ethanol. The chemical structures of compounds **2-5** would be established on the basis of micro analytical and spectroscopic data. The newly synthesized compounds **5** will be evaluated for their antimicrobial activity.

Importance of the study and published papers:

The literature survey reveals that isoxazole have been used as synthon for synthesis of a variety of heterocylces by cyclocondensation and by new synthetic methodologies, which have been found to possess promising pharmacological properties. In view of this, in the present investigation, a number of isoxazole derivatives have been utilized to build different heterocyclic rings on isoxazole moiety in order to evaluate their biological activity.

Published papers:

1. Synthesis and antimicrobial activity of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazines.
   R. Sanjeev, K. Thirupathaiah and E. Rajanarendar
   World journal of pharmacy and pharmocological sciences, vol. 6, issue 11, p.1248-1258.

2. Synthesis and anti-inflammatory and analgesic activity of 5-(1*H*-benzo[*d*]imidazolyl-2-yl)methyl-3-(3,5-dimethyl-4-isoxazolyl)-2-aryl-thiazolidin-4-ones.
   R. Sanjeev, K. Thirupathaiah and E. Rajanarendar
   Indian journal of chemistry, sec-B (CSIR), vol. 59B, 252-257, 2020.

Proposed work plan:

1. Synthesis of novel imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo-[2,3-*a*]pyrimidin-10-ones (5) would be accomplished by reaction of 3-amino-5-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl cyanides (2), with chloroacetyl chloride followed by treatment with aromatic primary amines and then with formaldehyde. The key intermediate *viz.,* 3-amino-5-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-6-yl-cyanides (2) was obtained by reaction of 3-amino-5-methyisoxazole (1) with aromatic aldehydes and malononitrile by a three-component one-pot process. The structures of newly synthesized compounds (5) will be established on the basis of spectral (IR, ^{1}H NMR, ^{13}C NMR, and Mass) and by micro analytical data. Compounds (5) will be evaluated for their *in vitro* antibacterial and antifungal data.

2. The reaction of 5-amino-2-methyl-7-aryl-7*H*-isoxazolo-[2,3-*a*]pyrimidin-6-yl cyanides 1 with triethyl orthoformate under refluxing condition produce the corresponding ethyl-*N*-(6-cyano-2-methyl-7-aryl-7*H*-isoxazolo[2,3-*a*]pyrimidin-5-yl)formimidates (2) in good yields. Compounds 2 on heating with hydrazine hydrate affords the cyclized products *viz.,* 4-hydrazinyl-5-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidines 3. The reaction of 3 with benzoyl acetonitrile in refluxing ethanol underwent cyclization to yield 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3-aryl-1*H*-pyrazol-5-amines (4). The synthesis of title compounds *viz.,* 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triaryl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitriles (5) would be achieved by trimolecular condensation of 4 with aromatic aldehydes and benzoyl acetonitrile in presence of $FeCl_3$ / basic Al_2O_3 in anhydrous ethanol. The chemical structures of compounds 2-5 would be established on the basis of micro analytical and spectroscopic data. The newly synthesized compounds 5 will be evaluated for their antimicrobial activity.

Proposed work plan/Formulation and structure of the study:

First year plan:

In the first year, I will collect the literature concerned to isoxazole chemistry and isoxazolyl heterocycles by visiting institutions like Indian institute of chemical Technology (IICT-CSIR) Hyderabad, and Indian Institute of Technology (IIT), Hyderabad and Central University Hyderabad. I will thoroughly search these libraries and will obtain a good content of literature pertaining to synthesis of isoxazole derivatives and different synthetic methodologies adopted and also biological activity associated with them. By coupling all these data, I will come to a conclusion to implement different new synthetic methodologies for the synthesis of proposed isoxazolyl heterocycles.

Second year plan:

I will start lab work during my second year plan. Based on the literature study, i will utilize new synthetic protocols for the synthesis of 2-methyl-7,11-diaryl-6,7,8,11-tetrahydro-10H-imidazo[1',5':1,2]pyrimido[4,5-*d*]isoxazolo[2,3-*a*]pyrimidin-10-ones and 1-(8-methyl-5-aryl-5*H*-isoxazolo[2,3-*a*]pyrimido[4,5-*d*]pyrimidin-4-yl)-3,4,6-triaryl-1*H*-pyrazolo[3,4-*b*] pyridine-5-carbonitriles. Like this some other heterocyclic substituted isoxazoles would be synthesized. All the newly synthesized compounds are charecterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies and by elemental analyses.

Third year plan:

I will screen all the title compounds synthesized during second year plan for antibacterial and antifungal activity. Different gram +Ve and gram –Ve bacterial strains and a variety of fungal strains would be utilized during this study, which would be obtained from different sources.

I will conclude the results obtained and also proceed for publishing the work in standard journals during this year.

At the end of this year, I will plan for writing my Ph.D. thesis work and submit the same to Swamy Ramanand Teerth Marathwada University, Nanded-431606, Maharasta, India for award of Degree of "Doctor of philosophy" (Ph.D.) in chemistry.

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